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Photoprotective responses in a brown macroalgae *Cystoseira tamariscifolia* to increases in CO_2 and temperature.

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ABSTRACT

Global warming and ocean acidification are increasingly affecting coastal ecosystems, with impacts that vary regionally depending upon local biogeography. Ocean acidification drives shifts in seaweed community dominance that depend on interactions with other factors such as light and nutrients. In this study, we investigated the photophysiological responses in the brown macroalgae species *Cystoseira tamariscifolia* (Hudson) Papenfuss with important structural role in the coastal Mediterranean communities. These algae were collected in the Cabo de Gata-Nijar Natural Park in ultraoligotrophic waters (algae exposed under high irradiance and less nutrient conditions) *vs*. those collected in the La Araña beach in oligotrophic waters (algae exposed at middle nutrient and irradiance conditions) in the Mediterranean Sea. They were incubated in mesocosms, under two levels of CO_2; ambient (400-500 ppm) and high CO_2 (1200-1300 ppm), combined with two temperatures (ambient temperature; 20ºC and ambient temperature + 4ºC; 24ºC) and the same nutrient conditions of the waters of the origin of macroalgae. Thalli from two sites on the Spanish Mediterranean coast were significantly affected by increases in pCO_2 and temperature. The carotenoids (fucoxanthin, violaxanthin and β-carotene) contents were higher in algae from oligotrophic than that from ultraoligotrophic water, i.e., algae collected under higher nutrient conditions respect to less conditions, increase photoprotective pigments content. Thalli from both locations upregulated photosynthesis (as F_v/F_m) at increased pCO_2 levels. Our study shows that ongoing ocean acidification and warming can increase photoprotection and photosynthesis in intertidal macroalgae.

INTRODUCTION

Atmospheric CO_2 levels have increased seawater temperatures by 0.13°C per decade over the last 50 years (IPCC 2104), causing a dieback in seaweeds at their warmest biogeographic limits (Harley et al., 2006, 2012; Wernberg et al., 2016). The increased concentration CO_2 atmospheric and their uptake is causing ocean acidification which increases the amount of carbon available to algae, and can stimulate their photosynthesis and growth (Johnson et al., 2015; Pajusalu et al., 2016), but it also lowers CO_3^{2-} levels which can cause dissolution of calcified algae (Newcomb et al., 2015).

Investigations into how global change will affect kelp forests and fucoid canopies are a priority as these habitats are of major ecological importance in temperate and cold-water regions of the planet (Brodie et al., 2014). Canopy-forming brown algae often proliferate in areas with naturally high levels of pCO_2 (Porzio et al., 2011; Roleda et al., 2012; Johnson et al., 2012; Linares et al., 2015). However, they have geographic range shifts in abundance over the past 50 years due to anthropogenic perturbations such as siltation, warming and increased nutrients levels (Díez et al., 2012; Strain et al., 2014; Yesson et al., 2015; Krumhansl et al., 2016; Wernberg et al., 2016). In the Mediterranean, the effect of ocean acidification on seaweed community composition is influenced by other factors such as light, nutrients and herbivory (Baggini et al., 2015; Celis-Plá el at., 2015, 2017). Phaeophytes such as *Cystoseira* spp., *Dictyota* spp., *Laminaria rodriguezii*, *Sargassum vulgare* and *Padina pavonica* increase in abundance near CO_2 seeps, where they may benefit from increased carbon availability (Porzio et al., 2011; Johnson et al., 2012; Baggini et al., 2014; Celis-Plá et al., 2015; Linares et al., 2015; Celis-Plá et al., 2017).

Cystoseira spp. are fucoid seaweeds that help maintain the structure and function of coastal ecosystems – they are used as indicators of high water quality in the Mediterranean (Bermejo et al., 2016; Celis-Plá et al., 2016). In this region, low nutrient availability limits algal photoprotection, photosynthesis and growth (Celis-Plá et al., 2016). High irradiance can stimulate an increase in photoprotective compounds, but only if the algae have sufficient nutrients (Abdala-Díaz et al., 2006). Intertidal macroalgae need to cope with large variations in light intensity and use photophysiological responses as photosynthesis activity, photoprotective compounds as carotenoids (violaxanthin, antheraxanthin and zeaxanthin) to help prevent damage to their photosystems (Goss and Jakob, 2010). And provide information about the damage, e.g., the maximum quantum yield of PSII (F_v/F_m), that use to determine photoinhibition and the physiological status of the fucoid macroalga (Figueroa et al., 2014, Celis-Plá et al. 2017).

Here, we investigated the interactive effects of pCO_2 (*ca.* 400-500 and *ca.* 1200-1300 ppm) and temperature (20ºC and 24ºC) predicted future temperature for the year 2100 (IPCC 2014), on *Cystoseira tamariscifolia* (Hudson) Papenfuss (Phaeophyceae, Fucales). The macroalgae were collected from Cabo de Gata-Nijar Natural Park (ultraoligotrophic waters) and La Araña beach, with less limited nutrient parts (oligotrophic waters) of the coast and maintained in mesocosms system with the same origin conditions to assess the projected effects of ocean acidification and warming. The Alboran Sea on Mediterranean coast of Spain is ultraoligotrophic in the southeast part with lower concentrations of nutrient and oligotrophic in the southwest, with increased nutrient levels due to local upwelling's (Ramírez et al., 2005; Mercado et al., 2007, 2012). We compared photophysiological responses of C. tamariscifolia collected from these two regions; our hypothesis was that the alga from oligotrophic waters would benefit in photoprotective compounds under elevated pCO_2 at ambient temperature when nutrient levels were sufficient, but that 4ºC warming would be detrimental.

MATERIALS AND METHODS

Sampling

Cystoseira tamariscifolia (Hudson) Papenfuss (Phaeophyceae, Fucales) specimens (Gómez-Garreta et al., 2001) were collected haphazardly from the low shore on 25 September 2013 in the Cabo de Gata-Nijar Natural Park (36º51'N; 2º6'W) and at La Araña Beach (36º42'N; 4º19'W) in the Mediterranean Sea. The Natural Park site is ultraoligotrophic, with lower concentrations of nitrate and phosphate than that in the La Araña site, (Table S1) that is classified as oligotrophic (Ramírez et al., 2005; Mercado et al., 2007, 2012).

Experimental conditions

After collection, 96 thalli in total (48 individuals from Natural Park and 48 individuals from La Araña) were transported to Malaga University where they were incubated for 28 days (after 48 hours of acclimation), in mesocosms with original conditions for ultraoligotrophic and oligotrophic macroalgae. The experimental system comprised 24 open tanks (14 L), with groups of three tanks connected in parallel to a 102 L tank. The mesocosms were held in 1000 L water baths (following the experimental set up described by Stengel et al. 2014). The thalli were incubated in four treatments: (1) ambient temperature (20ºC) x ambient CO_2 (*ca.* 400-500 ppm) ($ATxACO_2$), (2) ambient

temperature (20ºC) x high CO_2 (*ca.*1200-1300 ppm) ($ATxHCO_2$), (3) high temperature (24ºC) x ambient CO_2 (*ca.* 400-500 ppm) ($HTxACO_2$) and (4) high temperature (24ºC) x high CO_2 (*ca.*1200-1300 ppm) ($HTxHCO_2$), using 24 tanks in total with three replicate tanks for *C. tamariscifolia* from ultraoligotrophic and oligotrophic waters, respectively.

Temperature and DIC levels were controlled using a computer-operated control system (Aqua Medic T2001HC) in each header tank. The system automatically recorded one measurement every 15 min and was programmed to supply pure CO_2 via a solenoid valve as soon as the pH exceeded a threshold of 7.88 ± 0.01 in the header tanks (corresponding to *ca.* 1200-1300 ppm CO_2). The seawater carbonate system was monitored twice a week, taking water samples to measure the salinity, pH_{NBS} and total alkalinity (following methods given by Celis-Plá et al., 2017). The outdoor mesocosms were shaded using a mesh that reduced photosynthetically active radiation (PAR; 400-700 nm) by 35%, and UVA (320-400 nm) and UVB (280-320 nm) by 39%. Incident solar radiation was measured continuously in air using a UV-PAR Multifilter radiometer NILU-6 (Geminali). Levels of UVA and UVB radiation were calculated from the data of the different UV filters according to (Høiskar et al. 2003). Seawater was enriched with 2 µM nitrate (KNO_3) and 0.1 µM phosphate (KH_2PO_4) giving an N: P ratio of 20:1 for oligotrophic waters, and with 0.5 µM nitrate (KNO_3) and 0.1 µM phosphate (KH_2PO_4) giving an N:P ratio of 5:1 for ultraoligotrophic waters according to (Ramírez et al., 2005; Mercado et al., 2007, 2012) (Table S1).

Chlorophyll and carotenoid concentration and composition

Carotenoids and chlorophylls pigments content for fucoid macroalgae were determined to evaluate the capacity for acclimation, photoinhibition, photoprotection and vulnerability respect to the different irradiances and abiotic variables.

Pigments were extracted each week during the experimental period, 20 mg fresh weight from the apical parts of the algae, using 2 mL of 100% acetone and analysed using an ultra-high-performance liquid chromatographer (Shimadzu Corp., Kyoto, Japan) equipped with a photodiode array detector to measure peaks in the range 350-800 nm. After extraction, samples were centrifuged at 16200 g for 5 min (Sorvall Legend Micro 17, Thermo Scientific, Langenselbold, Germany) and then the extracts were filtered (0.22 µM nylon filters). The separation, was achieved with one column C-18 reversed phase (Shim-pack XR-ODS column; 3.0 × 75 mm, i.e.; 2.2 µm particle size; Shimadzu, Kyoto, Japan) protected by a guard column TR-C-160 K1 (Teknokroma, Barcelona, Spain). The

carotenoid composition was determined according to (García-Plazaola and Becerril 1999), with some modifications (Celis-Plá et al., 2015), using commercial standards (DHI LAB Products).

Photosynthetic activity as in vivo chlorophyll a fluorescence

In vivo chlorophyll *a* fluorescence associated with Photosystem II was determined using a portable pulse amplitude modulated (PAM) fluorometer (Diving-PAM, Walz GmbH, Germany). Thalli of the *C. tamariscifolia* were collected from natural populations (initial time) and monitored on day 7, 14, 21 and 28. In order to obtain rapid light curves (RLC) for each treatment in each week, apical parts of the macroalgae were put into 10 mL incubation chambers. The F_o (basal florescence) and F_m (maximal florescence) were measured after 15 minutes in darkness to obtain the maximum quantum yield (F_v/F_m) as a photoinhibition indicator being $F_v=F_m-F_o$, F_o the basal fluorescence of 15 min dark adapted thalli and F_m maximal fluorescence after a saturation light pulse of > 4000 µmol m^{-2} s^{-1} (Schreiber et al. 1995). According Celis-Plá et al. (2014a) were found no significant differences among the tested times (5, 15 and 30 min) for dark adapted in *Cystoseira tamariscifolia* and was selected 15 min for dark adapted as it is the most common dark exposure time found in the literature (Schreiber et al. 1995, Figueroa et al. 2014). The maximal quantum yield was used indicator of photoinhibition (F_v/F_m),

The Non-photochemical quenching (NPQ) was calculated according to Schreiber et al. (1995), as $NPQ = (F_m - F_{m'})/F_{m'}$. The maximal NPQ ($NPQ_{max}$) was obtained from the tangential function of NPQ versus irradiance function according to (Eilers and Peeters, 1998). NPQ was used as estimator of the photoprotection capacity.

Statistical analysis

Differences between physiological parameters in *C. tamariscifolia* were explored using a multivariate approach. A Principal Coordinates Analysis was performed for this purpose based on Euclidean distance using PERMANOVA+ for PRIMER6 package. This procedure calculates the percentage variation explained by each of the axes in the multidimensional scale. The overlay of the vectors onto the PCA was performed using Spearman correlation (Anderson, 2008).

The effects of the combined treatments on the photosynthetic activity and pigment contents of *C. tamariscifolia* were assessed using analysis of variance (ANOVA). This test was performed for *C. tamariscifolia* including time, temperature, pCO_2 and origin of

the population (locations) as categorical factors. Student Newman-Keuls tests (SNK) were performed after significant ANOVA interactions (Underwood, 1997). The homogeneity of variance of all data was confirmed by using Cochran tests and by visual inspection of the residuals (Underwood, 1997). Analyses were carried out using SPSS v.23 (IBM, USA).

RESULTS

The average daily-integrated irradiance for the experimental period was 4238 kJ m^{-2} for PAR, 329 kJ m^{-2} for UVA and 22 kJ m^{-2} for UVB. The seawater temperature was 19.95 ± 0.15 ºC in ambient temperature treatments and 23.91 ± 0.01ºC in the high temperature treatments (mean ± SE, n = 2232) (Table 1). The mean pH during the experimental period were 8.23 ± 0.01 in ATxACO_2, and 7.88 ± 0.01 in ATxHCO_2, 8.22 ± 0.01 in HTxACO_2 and 7.88 ± 0.01 in HTxHCO_2 treatments (Table 1), (calculated following methods given by Celis-Plá et al., 2015).

Principal coordinates analysis (Fig. 1) shows that at the end of the experiment, there was a positive correlation of the first axis (76.2% of total variation) with maximal quantum yield (F_v/F_m) being highest in samples from ultraoligotrophic waters for high temperature treatments under ambient and elevated pCO_2 (Cabo de Gata-Nijar Natural Park, L1). As well as, in oligotrophic waters samples (La Araña, L2), that were cultured at elevated temperature and ambient pCO_2. The chlorophylls a (as Chl*a*), violaxanthin (Viola), fucoxanthin (Fuco) and β-Carotene (β-Caro) were highest in samples collected in oligotrophic waters (L2) and grown at high temperature with ambient and high pCO_2 conditions. In contrast, maximal non-photochemical quenching (NPQ_{max}) and chlorophylls c (as Chl*c*) were higher in ambient temperature samples (Fig. 1)

Chlorophylls and Carotenoids

Chlorophyll *a* and *c* in all treatments had a significant interaction between time, temperature and CO_2 (p<0.01) (Fig. 2 and Table S2). The Student Newman-Keuls tests (SNK) revealed no clear differences between treatments but an overall trend of decline with time. Significant quantities of the fucoxanthin, violaxanthin and β-carotene (Tables 2 and S2) were detected in all treatments but only traces of antheraxanthin, lutein and zeaxanthin were found (data not shown).

Fucoxanthin content was affected by time, temperature and location, whereas violaxanthin was affected by the interaction between time, temperature and pCO_2 levels

(Tables 2 and S2). Fucoxanthin was higher after the experimental period for both locations, but in algae collected from oligotrophic waters, this pigment increased in respect to the ultraoligotrophic waters. Violaxanthin content was higher at the end the experimental period, in oligotrophic and ultraoligotrophic waters, but in oligotrophic waters, the violaxanthin was higher in respect to the other location. β-carotene content was significantly affected by time x pCO_2 conditions (Tables 2 and S2), it increased under increased pCO_2 conditions in thalli collected from oligotrophic waters (Tables 2 and S2).

Photosynthetic responses

Maximal quantum yield (F_v/F_m) as an indicator of photoinhibition varied significantly depending on time, temperature and CO_2 ($p<0.01$) (Table S3). F_v/F_m increased in samples collected in ultraoligotrophic waters under ambient pCO_2 conditions with ambient temperature, in addition in high temperature, the F_v/F_m increases in both pCO_2 levels (Fig. 4). Maximal non-photochemical quenching (NPQ_{max}), had interactive effects between time and temperature ($p<0.01$) (Table S3). The NPQ_{max} increased under high temperature in all thalli, irrespective of collection site and was highest at ambient temperature conditions independent of the pCO_2 levels (Fig. 5).

DISCUSSION

In this study, we show benefits of increased of the levels of dissolved inorganic carbon (DIC). Elevated CO_2 produces an increase in the photosynthetic yield and photoprotective compounds in *Cystoseira tamariscifolia* in the mesocosms system after several day incubation, confirming expected benefits of ocean acidification already reported for brown macroalgae (Cornwall et al. 2012; Bender et al. 2014; Celis-Plá et al. 2017). We show that these benefits in photophysiological responses were more rapid in fucoid *Cystoseira tamariscifolia* collected and grown in oligotrophic waters than ultraoligotrophic conditions. This highlights the fact that the effects of climate change and acidification on canopy-forming brown algae can be expected to differ depending on coastal water type. In ultraoligotrophic waters, levels of nutrients such as nitrate and orthophosphate are much lower than in oligotrophic waters, and this is coupled with the fact that irradiance is higher than in oligotrophic waters due to the high water transparency because the low phytoplankton productivity (Ramírez et al., 2005; Mercado et al., 2007, 2012). Increases in pCO_2 can boost algal growth in carbon-limited taxa (Cornwall et al. submitted) but only if sufficient nutrients and light are available to do so (Celis-Plá et al., 2015, 2017).

The carotenoid responses and other pigment contents were lowest in thalli that had been collected from ultraoligotrophic waters, suggesting that they were less able to invest in accumulation of biocompounds or other photoprotection system than thalli collected from a site where more nutrients were available (Stengel et al., 2014; Celis-Plá et al., 2014b, 2016). The carotenoid contents as; fucoxanthin, violaxanthin and β-carotene contents increased in those elevated pCO_2 treatments and temperature ambient under oligotrophic waters, i.e., waters with more nutrients contents. This corroborates the findings of Celis-Plá et al. (2015), who showed that *Cystoseira compressa* had higher concentrations of Chl*a*, photoprotectors compounds as phenols and fucoxanthin in nutrient enriched waters under high CO_2 conditions. Here we show that ambient temperature and elevated pCO_2 conditions can benefit algae collected in ultraoligotrophic waters. Many reviews concur that non-calcareous macroalgae production may increase due to beneficial effects of ocean acidification on photosynthesis, as long as the effects of warming and other stressors are not limiting (Harley et al., 2012; Koch et al., 2013; Kroeker et al., 2013). After one month, photoprotective contents, as carotenoids were higher in thalli collected from oligotrophic waters than those collected from ultraoligotrophic waters under high pCO_2 with ambient temperature.

Goss and Jakob (2010) indicated that the xanthophyll cycle related to NPQ represents an important photoprotection mechanism in plant cells. Demmig-Adams and Adams (2006) and García-Mendoza and Colombo-Pallota (2007) suggest more photoprotection, when increased the violaxanthin content, which is involved in photoprotection via the xanthophyll cycle (Demmig-Adams and Adams, 2006; García-Mendoza and Colombo-Pallota, 2007). In this study, showed in *C. tamariscifolia* important significant quantities of violaxanthin showed a differences in algae's from la Araña *vs* Cabo de Gata -Nijar, as responses of the photoprotection, in addition, a higher non-photochemical quenching, in the alga from La Araña in ambient temperature with ambient and higher CO_2 conditions. Responses of the xanthophyll cycle could reflect a regulatory and photoprotective response that down-regulates the delivery of excitation energy into the electron-transport chain to match the rates at which products of electron transport can be consumed in these algae (Demmig-Adams and Adams, 2006). García-Mendoza and Colombo-Pallota (2007) showed in brown algae *Macrocystis pyrifera* important ecophysiological responses of the photoprotection, a higher non-photochemical quenching, in the surface of the blades when the macroalgae were exposed to saturating light conditions. This thermal dissipation is measured as non-photochemical PSII fluorescence quenching (NPQ) is triggered by

301 the trans-thylakoidal proton gradient (ΔpH) along the thylakoid membrane that provides
302 energy for the synthesis of ATP by the ATP-synthase complex and zeaxanthin (ZEA)
303 synthesized through the xanthophyll cycle (Gilmore and Björkman, 1994; García-
304 Plazaola et al., 2012).
305 In this study, we found significant quantities of the violaxanthin was detected as
306 carotenoids involved xanthophyll cycle, or cycle with the corresponding formation of the
307 zeaxanthin (Z), but only traces of zeaxanthin. The activity of the xanthophyll or
308 violaxanthin (V-), cycle with the corresponding formation of zeaxanthin (Z) (Demmig-
309 Adams and Adams, 2006). Maximal non-photochemical quenching (NPQ_{max}) decreased
310 in enriched pCO_2 and high temperature conditions for algae collected in both localities
311 Elevated carbon content helps explain the dominance of these brown algae at a variety of
312 shallow water carbon dioxide seeps around Mediterranean coasts (Johnson et al., 2012;
313 Connell et al., 2013). Increased carbon availability is a direct stimulus for photosynthesis
314 (Mercado et al., 1998; Raven and Hurd, 2012) and can be used to make photoprotective
315 compounds that help algae dissipate excess thermal energy (Demmig-Adams and Adams,
316 2006; García-Plazaola et al., 2012).
317 At initial time, fucoxanthin content was about 8% higher in algae harvested from
318 ultraoligotrophic than that oligotrophic waters whereas the ratio between the main
319 carotenoid and chlorophyll (fucoxanthin: Chl*a*) was still higher (about 33.0%). After 28
320 days, however, the highest increase was produced in oligotrophic collected algae except
321 in high temperature with high CO_2 levels. However, experimental period submitted to
322 different pCO_2 and temperature treatments, the fucoxanthin/Chl*a* ratio was similar in
323 algae collected from both locations, i.e., 0.38-0.39 except in high temperature with high
324 pCO_2, i.e., 0.41 in ultraoligotrophic and 0.35 oligotrophic waters. Thus, the
325 fucoxanthin/Chl*a* ratio were favourable under the increase of both pCO_2 levels and
326 temperature in ultraoligotrophic than that oligotrophic harvested macroalgae. The high
327 proportion of photoprotective carotenoid (Goss and Jakob 2010) respect to chlorophyll
328 was expected since the penetration of both PAR and UVR (Figueroa and Gómez 2001) is
329 higher in ultraoligotrophic compared to oligotrophic waters due to its lowest turbidity and
330 high transparency (Mercado et al., 1998; Figueroa and Gómez, 2001). The high
331 proportion of the pigment content in *C. tamariscifolia* showed a high photoacclimation
332 in algae collected from ultraoligotrophic waters. We suggest that the decrease of the
333 carotenoids in ultraoligotrophic waters could be compensated by the other
334 photoprotectors, such as phenolic compounds. Celis-Plá et al. (2017) showed higher

concentration of phenolic compounds and antioxidant activity in algae collected from ultraoligotrophic waters, under high pCO_2 with ambient temperature, these suggest the increase of phenolic compounds under elevated pCO_2 could increase the photoprotection of *C. tamariscifolia* in future scenario of ocean acidification. The polyphenolic compounds are not only UV screen photoprotectors but also they have antioxidant capacity too (Celis-Plá et al., 2016). Thus, they can effective photoprotectors in waters with high UV penetration as Abdala-Díaz et al. (2006) showed previously in a yearly study in Cabo de Gata-Nijar Natural Park, ultraoligotrophic waters.

CONCLUSIONS

Elevated pCO_2 allowed *C. tamariscifolia* to up-regulate both photosynthetic yields and the production of photoprotective compounds. Our study shows that ocean acidification can interact with temperature and have beneficial effects on the accumulation of photoprotective carotenoids, as well as stimulating algal photosynthesis. We show that *C. tamariscifolia* is able to benefit from an increase in pCO_2 levels, rapidly changing their photoprotective composition and photophysiological responses, but the effects will depends upon interactions with other physicochemical parameters such as nutrient availability. Long-term experiments monitoring the effects of climate change on seaweeds in both tanks and *in situ* are necessary to know the vulnerability and adaptation capacity of primary producers in coastal habitats. *In vivo* chlorophyll *a* fluorescence is proving to be a useful tool in evaluations of the physiological status of algae under different climate change scenarios, of ocean acidification. The benefits of the ocean acidification for fucoids in the Spanish coast will be depend on there being enough nutrients and light in the intertidal communities. As well as, are not exceeded the thermal tolerances of their distribution limits.

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Table 1 Temperature, pH, pCO_2 (ppm) and Total Alkalinity (µmol kg^{-1}) in mesocosms system in four treatments. $ATxACO_2$ (ambient temperature, 20ºC x ambient CO_2, *ca.* 400-500 ppm), $ATxHCO_2$ (ambient temperature, 20ºC x high CO_2, *ca.*1200-1300 ppm), $HTxACO_2$ (high temperature, 24ºC x ambient CO_2, *ca.* 400-500 ppm) and $HTxHCO_2$ (high temperature, 24ºC x high CO_2, *ca.*1200-1300 ppm) (mean values ± SE).

	$ATxACO_2$	$ATxHCO_2$	$HTxACO_2$	$HTxHCO_2$
Temperature (°C)	19.8 ± 0.01	20.1 ± 0.01	23.9 ± 0.02	23.9 ± 0.01
pH_{NBS}	8.34 ± 0.01	7.88 ± 0.01	8.22 ± 0.01	7.88 ± 0.01
pCO_2 (µatm)	455.6 ± 11.9	1264.1 ± 30.2	509.8 ± 7.8	1274.8 ± 17.9
Total Alkalinity (µmol kg^{-1})	2431 ± 11.99	3585 ± 14.16	3059 ± 13.45	3793 ± 5.31

Table 2 Fucoxanthin, violaxanthin and β-carotene (μg g^{-1} DW) of *Cystoseira tamariscifolia* at the start of the experiment (*It*) and after 7, 14, 21 and 28 days of incubation, for ultraoligotrophic waters (Cabo de Gata-Nijar Natural Park), oligotrophic waters (La Araña beach) and 4 treatments. $ATxACO_2$ (ambient temperature, 20ºC x ambient CO_2, *ca.* 400-500 ppm), $ATxHCO_2$ (ambient temperature, 20ºC x high CO_2, *ca.*1200-1300 ppm), $HT*ACO_2$ (high temperature, 24ºC x ambient CO_2, *ca.* 400-500 ppm) and $HTxHCO_2$ (high temperature, 24ºC x high CO_2, *ca.*1200-1300 ppm) (mean values ± SE).

		Cystoseira tamariscifolia									
		Ultraoligotrophic waters					**Oligotrophic waters**				
		It	*7 d*	*14 d*	*21 d*	*28 d*	*It*	*7 d*	*14 d*	*21 d*	*28 d*
Fucoxanthin	$ATºCxACO_2$	206.3 ± 78.1	316.8 ± 102.5	386.7 ± 52.7	386.5 ± 71.3	426.5 ± 14.7	191.2 ± 31.5	352.8 ± 37.7	527.8 ± 112.1	536.1 ± 101.5	904.1 ± 82.2
	$ATºCxHCO_2$		247.3 ± 30.1	429.2 ± 17.3	417.6 ± 14.1	321.5 ± 34.9		386.1 ± 89.1	443.7 ± 61.3	547.8 ± 84.3	801.1 ± 159.5
	$HTºCxACO_2$		230.5 ± 37.5	246.9 ± 36.8	380.2 ± 77.1	434.1 ± 43.6		443.2 ± 81.1	573.8 ± 44.1	477.3 ± 89.9	830.1 ± 131.8
	$HTºCxHCO_2$		361.8 ± 65.2	437.7 ± 73.7	276.7 ± 33.4	437.3 ± 56.1		558.6 ± 110.4	626.1 ± 99.6	478.6 ± 58.3	401.9 ± 93.9
Violaxanthin	$ATºCxACO_2$	29.5 ± 6.5	65.3 ± 4.5	49.9 ± 10.5	57.2 ± 5.7	64.6 ± 0.7	46.4 ± 13.4	44.3 ± 15.7	68.9 ± 11.2	81.7 ± 14.1	118.2 ± 6.1
	$ATºCxHCO_2$		23.8 ± 8.1	52.3 ± 7.3	57.7 ± 2.1	45.1 ± 5.1		33.3 ± 2.7	69.5 ± 8.7	82.6 ± 11.2	113.2 ± 21.6
	$HTºCxACO_2$		26.7 ± 3.4	38.1 ± 4.8	52.4 ± 9.5	63.2 ± 5.1		22.7 ± 4.7	69.1 ± 3.1	65.1 ± 8.5	111.1 ± 13.6
	$HTºCxHCO_2$		51.4 ± 9.7	58.1 ± 6.1	38.7 ± 4.3	60.7 ± 7.6		90.4 ± 23.7	82.5 ± 20.8	72.5 ± 11.3	68.1 ± 12.6
β- carotene	$ATºCxACO_2$	21.5 ± 3.4	53.9 ± 3.7	56.4 ± 11.3	70.5 ± 3.2	74.6 ± 3.4	61.7 ± 15.6	63.4 ± 16.3	103.1 ± 13.5	113.1 ± 15.1	107.4 ± 7.8
	$ATºCxHCO_2$		47.1 ± 15.9	83.2 ± 17.7	83.9 ± 10.1	60.1 ± 8.6		119.1 ± 23.8	94.1 ± 15.5	96.3 ± 7.6	107.6 ± 11.5
	$HTºCxACO_2$		48.2 ± 10.1	69.5 ± 15.9	69.1 ± 2.1	91.1 ± 15.1		64.1 ± 5.8	88.8 ± 4.2	81.6 ± 2.4	113.8 ± 13.4
	$HTºCxHCO_2$		43.1 ± 4.1	63.9 ± 3.7	156.4 ± 7.6	73.6 ± 0.7		76.7 ± 12.1	92.2 ± 11.9	77.3 ± 11.5	78.4 ± 4.3
Fucoxanthin /Chla	$ATºCxACO_2$	0.14 ± 0.03	0.11 ± 0.03	0.43 ± 0.01	0.40 ± 0.01	0.38 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.36 ± 0.01	0.37 ± 0.01	0.39 ± 0.02
	$ATºCxHCO_2$		0.14 ± 0.01	0.42 ± 0.01	0.40 ± 0.01	0.39 ± 0.01		0.12 ± 0.01	0.34 ± 0.01	0.37 ± 0.01	0.38 ± 0.01
	$HTºCxACO_2$		0.13 ± 0.01	0.39 ± 0.01	0.42 ± 0.01	0.39 ± 0.01		0.20 ± 0.02	0.40 ± 0.02	0.40 ± 0.03	0.39 ± 0.01
	$HTºCxHCO_2$		0.13 ± 0.01	0.40 ± 0.01	0.44 ± 0.02	0.41 ± 0.02		0.16 ± 0.03	0.40 ± 0.02	0.39 ± 0.01	0.35 ± 0.01

Figure Captions

Figure 1 Principal component analysis of *Cystoseira tamariscifolia* respect to variables; maximal quantum yield (F_v/F_m), maximal non-photochemical quenching (NPQ_{max}), Chlorophylls *a* and *c* (Chl*a* and Chl*c*), carotenoids pigments; fucoxanthin (Fuco), violaxanthin (Violo) and β-carotene (β-Caro). For ultraoligotrophic (L1) and oligotrophic (L2) waters, after exposure to four treatments, $ATxACO_2$ (ambient temperature, 20ºC x ambient CO_2, *ca.* 400-500 ppm), $ATxHCO_2$ (ambient temperature, 20ºC x high CO_2, *ca.*1200-1300 ppm), $HTxACO_2$ (high temperature, 24ºC x ambient CO_2, *ca.* 400-500 ppm) and $HTxHCO_2$ (high temperature, 24ºC x high CO_2, *ca.*1200-1300 ppm).

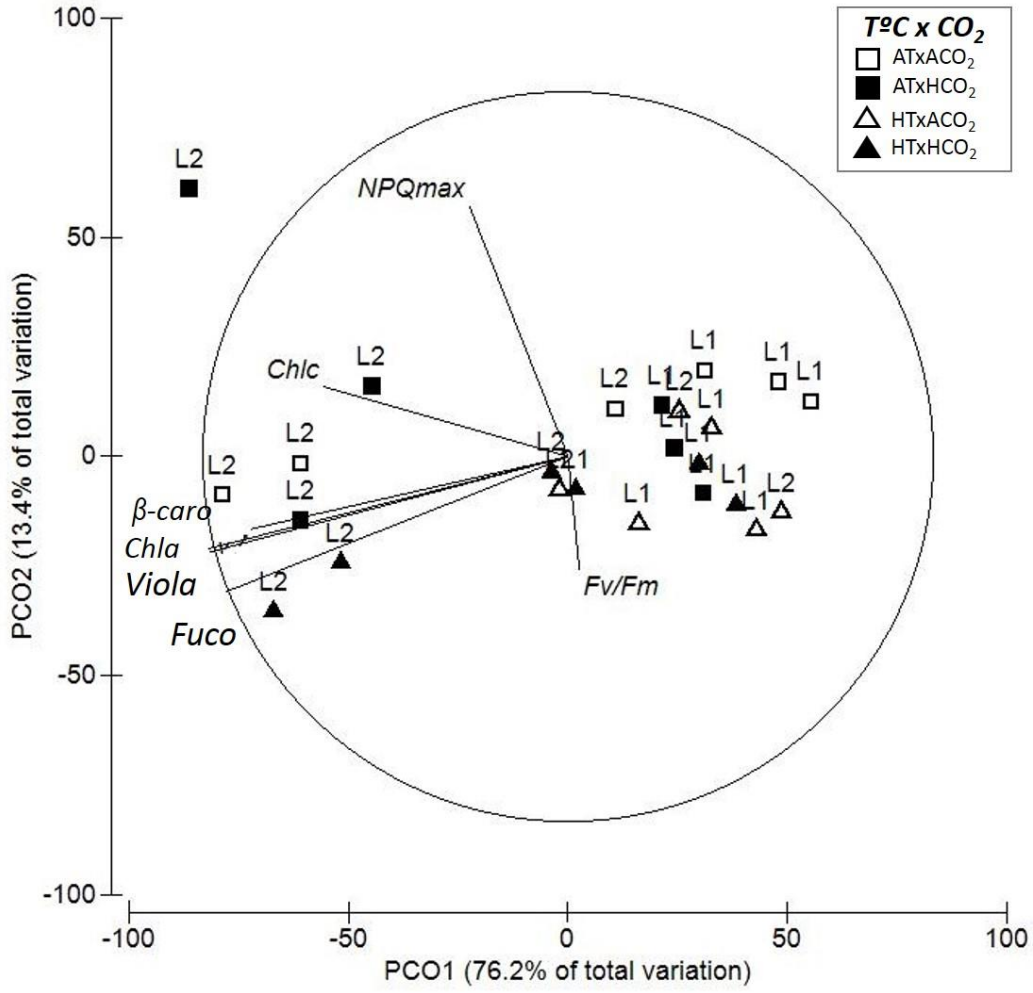
Figure 2 Chlorophyll *a* (mg g^{-1} DW), a) *Cystoseira tamariscifolia* from ultraoligotrophic waters and b) oligotrophic waters, after exposure to four treatments. Ambient TºC (20ºC) x ambient CO_2 (*ca.* 400-500 ppm), ambient TºC (20ºC) x high CO_2 (*ca.*1200-1300 ppm), high TºC (24ºC) x ambient CO_2 (*ca.* 400-500 ppm) and high TºC (24ºC) x high CO_2 (*ca.*1200-1300 ppm). Lower-case letters denote significant differences after SNK test ($p<0.05$).

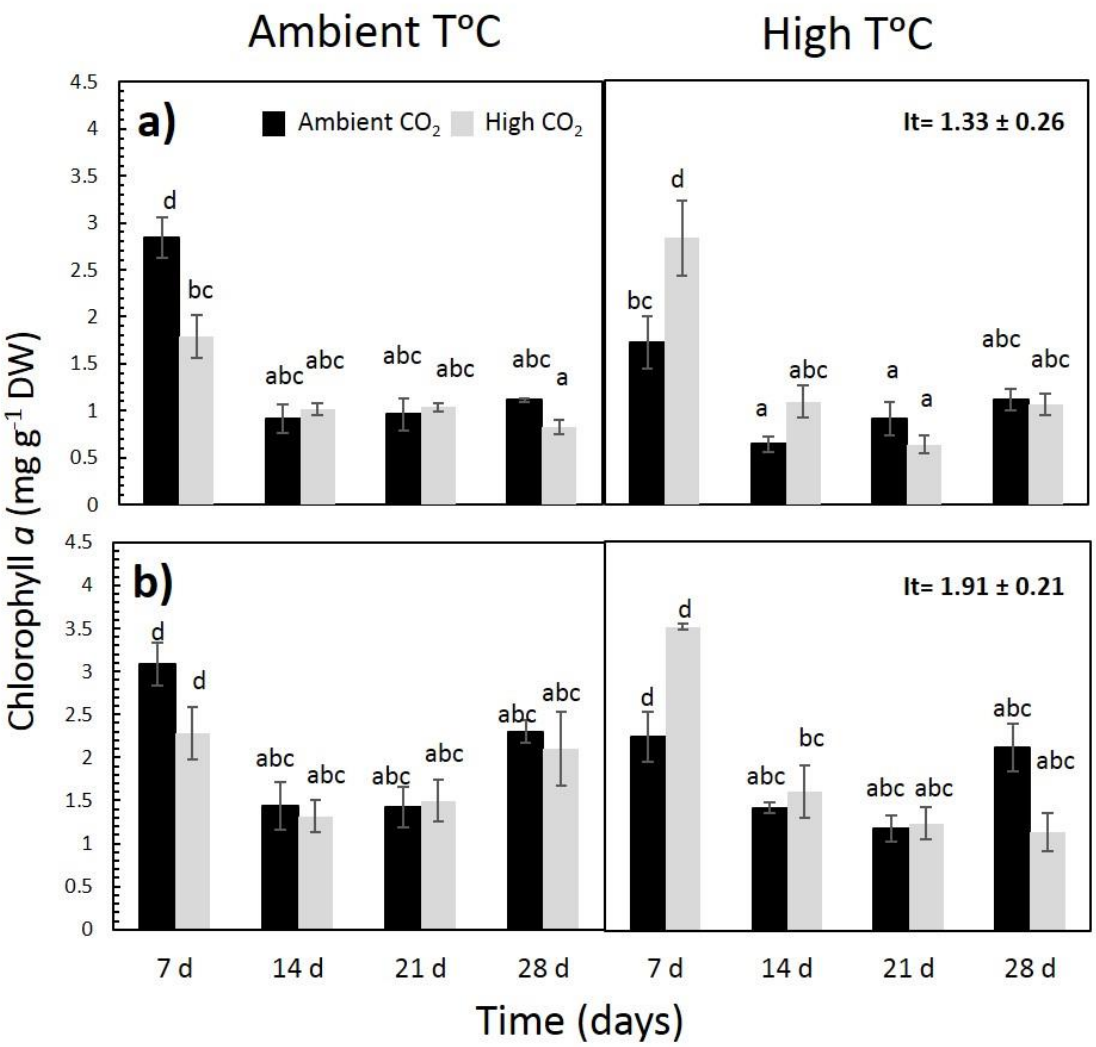
Figure 3 Chlorophyll *c* (µg g^{-1} DW), a) *Cystoseira tamariscifolia* from ultraoligotrophic waters and b) oligotrophic waters, after experimental period and four treatments. Ambient TºC (20ºC) x ambient CO_2 (*ca.* 400-500 ppm), ambient TºC (20ºC) x high CO_2 (*ca.*1200-1300 ppm), high TºC (24ºC) x ambient CO_2 (*ca.* 400-500 ppm) and high TºC (24ºC) x high CO_2 (*ca.*1200-1300 ppm). Lower-case letters denote significant differences after SNK test ($p<0.05$).

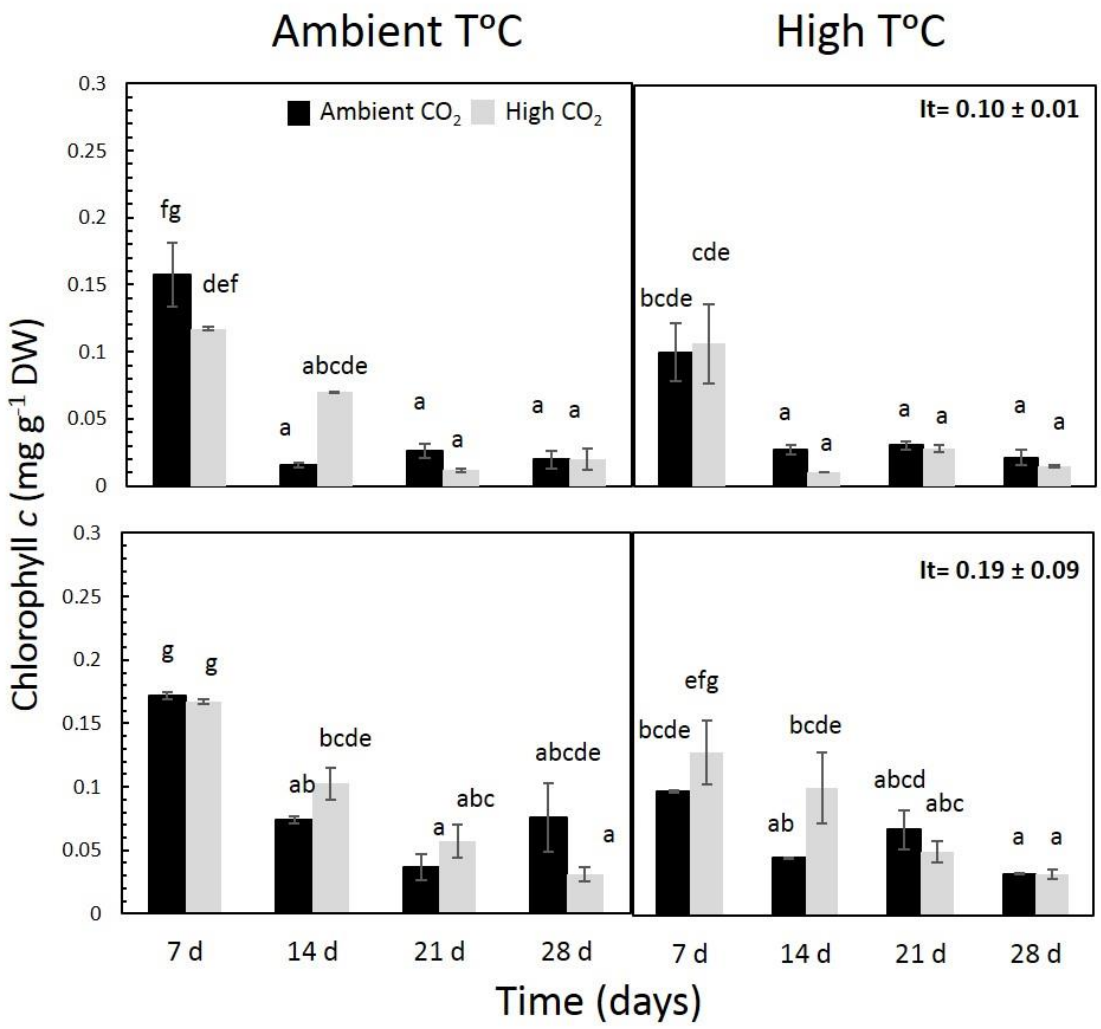
Figure 4 Maximal quantum yield (F_v/F_m), a) *Cystoseira tamariscifolia* from ultraoligotrophic waters and b) oligotrophic waters (La Araña beach), after experimental period and four treatments. Ambient TºC (20ºC) x ambient CO_2 (*ca.* 400-500 ppm), ambient TºC (20ºC) x high CO_2 (*ca.*1200-1300 ppm), high TºC (24ºC) x ambient CO_2 (*ca.* 400-500 ppm) and high TºC (24ºC) x high CO_2 (*ca.*1200-1300 ppm). Lower-case letters denote significant differences after SNK test ($p<0.05$).

Figure 5 Maximal non-photochemical quenching (NPQ_{max}), a) *Cystoseira tamariscifolia* from ultraoligotrophic waters and b) oligotrophic waters after experimental period and four treatments. Ambient TºC (20ºC) x ambient CO_2 (*ca.* 400-500 ppm), ambient TºC

(20ºC) x high CO_2 (*ca.*1200-1300 ppm), high TºC (24ºC) x ambient CO_2 (*ca.* 400-500 ppm) and high TºC (24ºC) x high CO_2 (*ca.*1200-1300 ppm). Lower-case letters denote significant differences after SNK test ($p<0.05$).


TºC x CO2
ATxACO2
ATxHCO2
HTxACO2
HTxHCO2
NPQmax
Chlc
β-caro
Chla
Viola
Fuco
Fv/Fm
PCO2 (13.4% of total variation)
PCO1 (76.2% of total variation)
100
50
0
-50
-100
-100
-50
0
50
100


Ambient T°C
High T°C
a)
b)
Ambient CO2
High CO2
It= 1.33 ± 0.26
It= 1.91 ± 0.21
Chlorophyll a (mg g-1 DW)
Time (days)
7 d
14 d
21 d
28 d


Ambient T°C
High T°C
Ambient CO_2
High CO_2
It= 0.10 ± 0.01
It= 0.19 ± 0.09
Chlorophyll c (mg g^{-1} DW)
Time (days)
7 d
14 d
21 d
28 d
0
0.05
0.1
0.15
0.2
0.25
0.3
fg
def
a
abcde
a
a
a
a
bcde
cde
a
a
a
a
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g
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bcde
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abc
abcde
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bcde
efg
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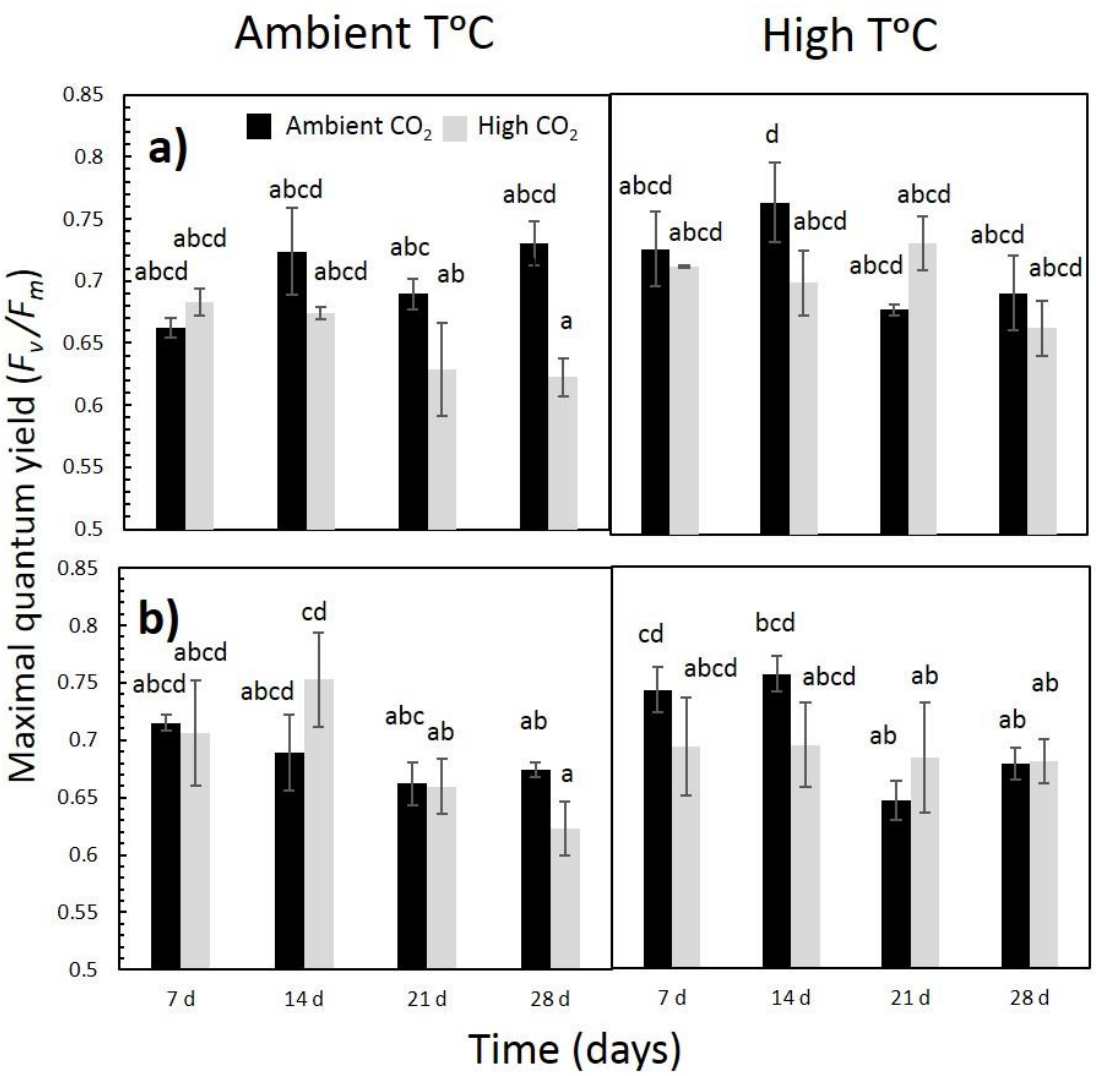
bcde
abcd
abc
a
a


Ambient T°C
High T°C
a)
b)
Ambient CO2
High CO2
Maximal quantum yield (Fv/Fm)
0.85
0.8
0.75
0.7
0.65
0.6
0.55
0.5
abcd
abcd
abcd
abcd
abc
ab
abcd
a
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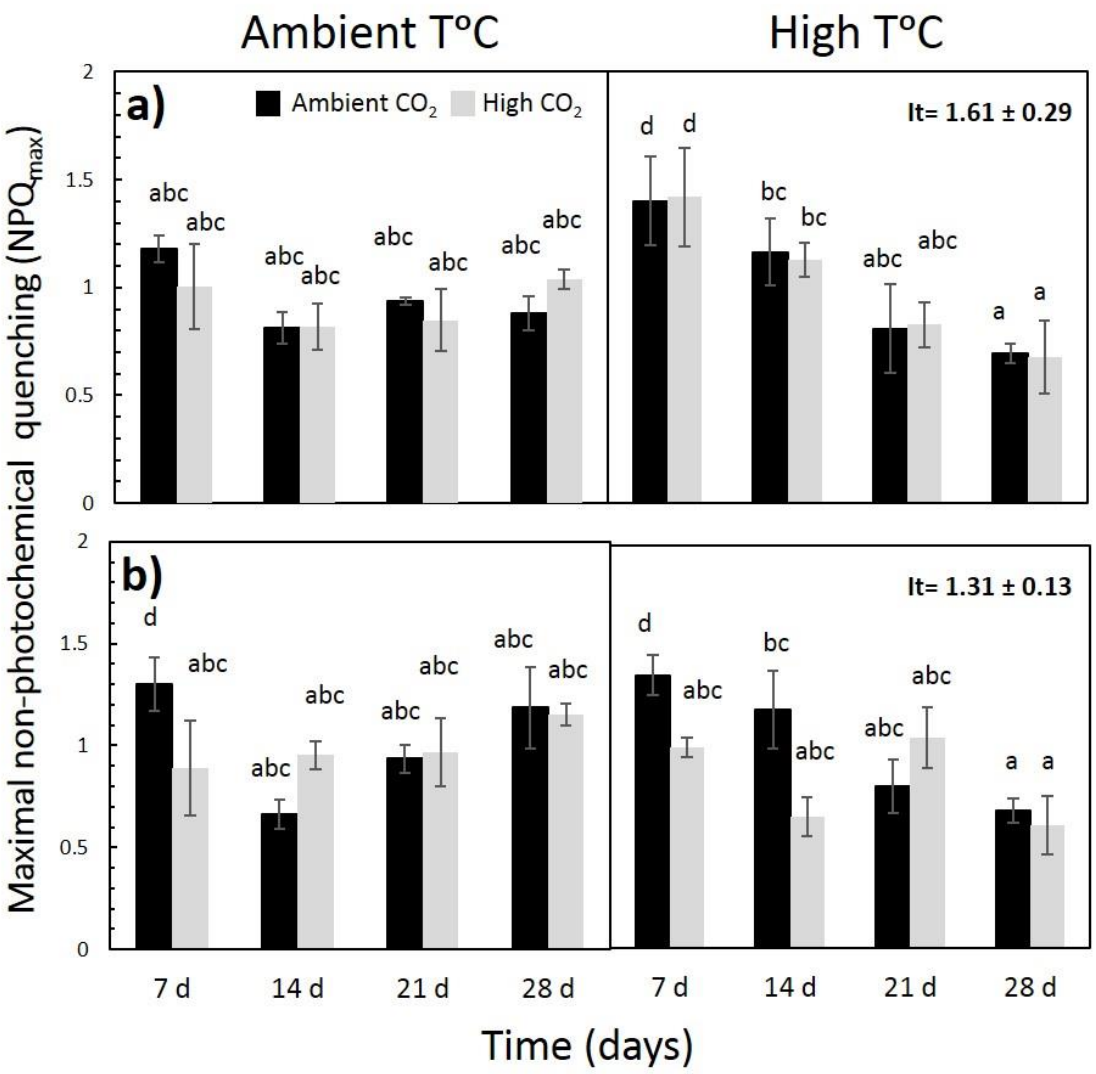
21 d
28 d
7 d
14 d
21 d
28 d
Time (days)


Ambient T°C
High T°C
a)
b)
Ambient CO2
High CO2
It= 1.61 ± 0.29
It= 1.31 ± 0.13
Maximal non-photochemical quenching (NPQmax)
Time (days)
7 d
14 d
21 d
28 d